OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

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P.P. 6F1675. Request for tolerance for the residues of Bidrin(R) Insecticide in or on wheat and sorghum grain of 0.05 ppm.

Edwin R. Budd, Toxicology Branch, Registration Division.

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Frank Gee, PM# 16, Registration Division.

Pesticide Petition No. 6F1675

Petitioner: Shell Chemical Company, San Ramon, California.

Trade Name: Bidrin(R)

Chemical Names: dimethyl phosphate of 3-hydroxy-N, N-dimethyl-cis-crotonamide;
3-(dimethoxy phosphinyloxy)-N,N-dimethyl-cis-crotonamide;
SD-3562.

Action Requested: Establish tolerance for residues of the insecticide dimethyl phosphate of 3-hydroxy-N,N-dimethyl-cis-crotonamide in or on wheat and sorghum grain of 0.05 ppm.

Recommendation: Toxicology Branch recommends against establishment of the requested tolerance at this time due to the lack of a second required oncogenic potential evaluation study. (See "Present Action" at the end of this review.)

Related Petitions: 7G0571, 1F1062.

Established Tolerances: In or on cottonseed at 0.05 ppm. (40 CFR 180.299)

Structural Formula:

This cis (or alpha) isomer is defined as Bidrin Insecticide. Note that Bidrin contains a typical organophosphate structure. It is listed as a cholinesterase-inhibiting pesticide (40 CFR 180.3).

Use: Agricultural insecticide.

Inert ingredient information may be entitled to confidential treatment

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Requested Application: Mix the required amount of Bidrin in sufficient water to provide uniform coverage of foliage by ground spraying or by aerial application. Wheat (Texas, Oklahoma and Kansas only) - 4.0 to 8.0 fluid ounces per acre, repeat as necessary, do not apply within 45 days of harvest or grazing.

Sorghum (Texas, Oklahoma, Colorado and Kansas only) - 4.0 fluid ounces per acre, do not apply within 45 days of harvest, do not apply after seed head emerges from boot, do not apply more than once per season.

Technical Product: Technical Bidrin(R) Insecticide, contains two isomers of Bidrin. The cis or alpha isomer is defined as Bidrin(R) Insecticide. Typical components of technical Bidrin(R) Insecticide are shown below.

Component	% (W/W)
3-(dimethoxyphosphinyloxy-N,N-dimethyl-cis-crotonamide	87
3-(dimethoxyphosphinyloxy)-N,N-dimethyl-trans-crotonamide	6
N,N-dimethylacetoacetamide	1
N,N-dimethyl-2-chloroacetoacetamide	3
N,N-dimethyl-2,2-dichloroacetoacetamide	1
3-(dimethoxyphosphinyloxy)-2-chloro-N,N-dimethyl-crotonamide	1
Unidentified Impurities	1
	100%

Formulated Product: Bidrin(R) 8 Water Miscible Insecticide (EPA Reg. No. 201-274-AA)

Component	% (W/W)
Technical Bidrin(R) Insecticide	
Bidrin (cis isomer)	82
[redacted]	
	100%

Contains 8 lbs active ingredient per gallon
*cleared in 40 CFR 180.1001 (c)(d)

Background Toxicity Data: The following background toxicity data was taken directly from previous reviews as described below.

Reviewer	Petition Reviewed	Review Date
R. D. Coberly	P.P. 7G0571	4/13/67
R.T. Habermann	P.P. 7G0571	6/12/67
C.H. Williams	P.P. 1F1062	5/21/71
D. M. Reisa	P.P. 6F1675	1/14/76

The review dates following each toxicity study summary (below) indicate the specific review from which the data was summarized.

Acute Oral Toxicity		Review Date
✓Rats, technical	LD_{50}=22(19-25) mg/kg	4/13/67,6/12/67
✓Rats, technical	LD_{50}=12.8(9.1-15.7) mg/kg	4/13/67
✓Rats, in isopropyl alcohol	LD_{50}= 28.7 mg/kg	4/13/67
✓Rats, in dimethyl sulphoxide	LD_{50}=30.2 mg/kg	4/13/67
✓Mice,male	LD_{50}=15 mg/kg	6/12/67
✓Mice, in isopropyl alcohol	LD_{50}=20 mg/kg	4/13/67
✓Mice, in dimethyl	LD_{50}=40-50 mg/kg	4/13/67
Ducks	LD_{50}=2.5-5.0 mg/kg	4/13/67
Fowls	LD_{50}=10-12.5 mg/kg	4/13/67
✓Hens	LD50=7.4 mg/kg	5/21/74

Typical signs of organo phosphate intoxication: salivation, lacrimation, diarrhea, tremors and terminal convulsions.

Acute Dermal Toxicity

✓Rats, male, technical	LD_{50}136 mg/kg	4/13/67
✓Rats, female, technical	LD_{50}=111 mg/kg	4/13/67
✓Rabbits	LD_{50}=224(102-505)mg/kg	6/12/67

6 hour exposure; salivation, diarrhea, tremors, progressive loss of coordination, weakness, collapse, prostration and death.

Cattle	1 gallon of 0.5% spray (≈ 60 mg/kg) to head was a borderline systemic toxicity dose; 60 mg/kg (in water or glycerin) to back caused no symptoms; 12.5 mg/kg (in olive oil)	6/12/67

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Acute INhalation Toxicity		REview Date
	to back caused symptoms; full coverage with 0.25% spray caused no symptoms.	
Rats, male, technical, undiluted	LC_{50}=0.65 mg/l 1 hour exposure: restlessness, increased respiration, tremors, salivation, weakness, excessive defecation, dypsnea, convulsions and terminal coma during exposure and up to 2 hours post exposure. After exposure, animals which eventually survived recovered promptly.	4/13/67,6/12/67
Rats, male, aqueous solution of 38.2% Bidrin	LC_{20}=0.86 mg/l	4/13/67, 6/12/67
	Not lethal at 2 mg soln/l.	

Acute Subcutaneous Toxicity

Guinea pigs, technical	LD_{50}=45 mg/kg	5/21/71

Primary Eye Irritation

Rabbits	conjunctival erythema (grades 2-3) within 30 sec. Washing did not reduce erythema. Opacity (grade 2) within 10 min. Also, mild discharge (grade 1). Miosis at 7-8 min. All eyes appeared normal within 24 hours.	6/12/67

Primary Skin Irritation

None

Subacute Dermal Toxicity

Rabbits, 6.67% aqeous soln.	3 weeks; 0,20 and 40 mg/kg/day, 6 hours/day and 5 days/week. 2 mortalites (5 and 13 days) at 40 mg/kg/day following diarrhea and loss of body weight. Very mild skin irritation in a few animals, majority showed no dermal irritation. Other parameters examined were negative.	6/12/67

Subacute Inhalation Toxicity

Rats NEL=0.81 mg/liter of air 5/21/71

Subacute Feeding Toxicity Review Date

✓ Rats, 2 weeks Subacute LD_{50} ♂♀ ≈440 ppm 4/13/67,6/12/67
0,10, 30, 100, 250, 750, 2000 and 4000 ppm in diet for 2 weeks. All rats ≥ 750 ppm died <7 days. At remaining levels, erythrocyte, plasma, whole blood and brain cholinesterase activities were substantially inhibited. Weight gains in females at remaining levels were depressed.

✓ Rats, 12 weeks NEL=45 ppm (based on systemic effects only). 6/12/67
0,15,45 and 135 ppm in diet for 12 weeks. At 135 ppm, observed decreased body weight gains, increased spleen, heart and testes weights (males), and increased kidney weights (females)

✓ Rats, 12 weeks NEL = 0.5 ppm (based on cholinesterase effects) 6/12/67

0,0.5, 1.5 and 4.5 ppm in diet for 12 weeks. Whole blood cholinesterase activity was inhibited at 1.5 ppm (4-33%) and at 4.5 ppm (42-72%). Brain cholinesterase activity inhibition of borderline significance at 1.5 ppm and significantly decreased at 4.5 ppm. Recovery times (after cessation of test diet) were < 2-3 weeks.

✓ Dogs (beagles), 3 1/2 weeks NEL= 1.5 ppm 6/12/67
(based on cholinesterase effects)
0,0.5, 1.5 and 4.5 ppm in diet for 3 1/2 weeks. Whole blood cholinesterase activity was depressed at 4.5 ppm (72-76%). Erythrocyte activity was depressed at 4.5 ppm (77-86%) - but was not significant. Plasma activity ~~activity~~ was depressed at 4.5 ppm (30%).

✓ Dogs (beagles), 13 weeks 4/13/67, 6/12/67
NEL=50 ppm (based on systemic effects). NEL between 1 and 5 ppm (based on cholinesterase effects - 6/12/67). "CHI noted at all dosage levels (1.0-50 ppm)"-in 4/13/67 review.
0,1,5 and 50 ppm in diet for 13 weeks. 4 males and 4 females/group. No mortalities. At 50 ppm, cholinesterase activity was inhibited in erythrocytes (up to ♂♀30% at 7 and 10 weeks), in plasma (♂50% in males) and in brain (50%). At 1 and 5 ppm, plasma levels were significantly decreased (dose-related) at

[redacted]

6-8 weeks but were within normal limits at 13 weeks. All other parameters examined were essentially negative for effects of the test material.

Chronic Feeding Toxicity

Rats, 2 years 6/12/67, 5/21/71

NEL = 1 ppm (based on cholinesterase effects).
NEL = 10 ppm (based on systemic effects). 0,1,10 and 100 ppm in diet for 2 years. 25 males and 25 females/test group and 40 males and 40 females/control group. Examined general condition, food intake, survival, body weights, hematology, cholinesterase activity (erythrocytes, plasma and brain), gross necropsies, organ weights (11 organs) and histopathology in control and 100 ppm groups (21 tissues). Interim report at 1 year (6/12/67) At 100 ppm, ±60% of the test animals exhibited diarrhea and/or tremors at 1 week. Tremors occurred through the 4th week. Erythrocyte and plasma cholinesterase activities were decreased (dose-related) in 10 ppm and 100 ppm groups at 6, 13, 26 and 52 weeks.

Final report - Mortality (at 2 years) ranged from 63-72% in Bidrin groups and 28-58% in controls (both sexes). At 100 ppm, observed depression of growth, decreased frequency of slight hepatocellular vacuolation [presumably when compared to control] and increased severity of hepatocellular inflammatory changes. Plasma, erythrocyte and brain cholinesterase activities were decreased at 10 ppm and 100 ppm at 78 and 104 weeks. "The percent of total neoplasms was comparable in control and Bidrin-treated groups."

Dogs (beagles), 2 years 6/12/67/5/21/71

NEL = 1.6 ppm (based on erythrocyte cholinesterase).
NEL = 16 ppm (based on systemic effects).
0, 0.16, 1.6 and 16 ppm in diet for 106 weeks. An additional group started at about 52 weeks at 100 ppm. 4 males and 4 females in control group, 2 males and 2 females in 100 ppm group and 3 males and 3 females in remaining groups. Parameters examined included condition, food intake (?), body weights, survival, hematology, urinalyses, clinical chemistry determinations, cholinesterase activity (erythrocytes, plasma, and brain), blood pressures, EKG, heart rates, eye examinations, gross necropsies, organ weights (11 organs) and histopathology in control, 16 and 100 ppm groups (29 tissues) and in 0.16 and 1.6 ppm groups (9 tissues). Interim report at 1 year (6/12/67) At 100 ppm, observed diarrhea, slight tremors, salivation and body weight losses during first 3 weeks.
Final report - One female at 100 ppm was sacrificed at 31 weeks following a marked body weight drop. There were no other mortalities. Plasma cholinesterase activity was depressed at 1.6 (questionable), 16 and 100 ppm. Erhthrocyte cholinesterase activity was markedly inhibited at 16 and 100 ppm. Brain

increase of sarcolemmal nuclei in skeletal muscle and one female (sacrificed at 31 weeks) had degenerative changes in muscle, liver, kidney; and inflammatory changes in the pancreas and gastrointestinal tract.

Oncogenic Study

Rats, 2 years 6/12/67, 5/21/71

The 2 year chronic feeding study on rats described and summarized above includes a valid oncogenic evaluation. Results for oncogenicity were negative.

Reproduction Studies REview Date

Rats, one generation study 4/13/67, 6/12/67

NEL= 3.0 ppm (4/13/67). 0,0.3, 3.0 and 30.0 ppm in diet for 2 weeks preceding mating and continued thereafter for duration of study. First and second litters were obtained from same dams. At 3 weeks post-partum (second litters), dams and progeny were sacrificed and examined. No terata or effect on number of pups at any dosage level. At 30.0 ppm, there was a 10-15% decrease in fertility, a high mortality rate (80% for first mating and 93% for second mating-stillborns and deaths during nursing period) and the pups were small and appeared undernourished.

Rats, three generation study 6/12/67

NEL=2 ppm. 0,2, 5, 15 and 50 ppm in diet through 3 generations. 10 males and 20 females/group. Females of each generation produced 2 litters. Usual reproduction parameters examined. At 50 ppm, parents and offspring both exhibited weakness, emaciation and/or CNS effects; there were fewer litters and litter sizes were significantly smaller; and mortality of pups was so high that it was not possible to continue beyond the first generation. At 15 ppm, mortality of pups was significantly higher than in controls. At 5 ppm, in F_2a and F_2B litters, mortality of pups was significantly higher than in controls. No other changes attributable to the test material were observed.

Teratology Studies

Rats 6/12/67

0,50 and 100 ppm in diet for about 3 weeks preceding mating and continued thereafter until 10th to 17th day of pregnancy. At 100 ppm, the average implantation rate was decreased 26.5% compared to controls. At 50 ppm, 9.7% of the established pregnancies failed to develop (in controls, 1.1 % failed to develop). No terata were observed in any offspring. "It is suggested that Bidrin may interfere with implantation and placentation."

Rabbits

A teratology study on rabbits was submitted with this petition. See the section of this review entitled "New Studies submitted in this Petition" for a review of this study.

Neurotoxicity Studies

✓ Rats 4/13/67
0,5,10,20 and 40 ppm in diet for 6 weeks. Weekly whole blood cholinesterase activity determinations. At 5 ppm, 40-60% inhibition and 35% depression in brain cholinesterase activity at necropsy. Even greater inhibitions at higher dosage levels.

Dogs 5/21/71
NEL=1.5 ppm (for cholinesterase effects)
12 week study. Blood cholinesterase activity determinations.

Species (?), in vitro study 5/21/71
In vitro brain cholinesterase activity was inhibited up to to 50% by Bidrin, whereas other organophosphates (dichlorvos, ciodrin, chlorfenvinphos, SD 779 and paraoxon) inhibited cholinesterase activity up to 62-89%.

✓ Hens 5/21/71
Demyelination study employing single oral doses. No neurotoxic effects observed at 8-9 mg/kg.

Hens, White Leghorn, 12-18 months old 6/12/67
Approximately 1/10 of the oral LD_{50} (LD_{50}=7.4 mg/kg) was administered, in gelatin capsules, "on each of several days" for 3 weeks to 15 hens. Moderately severe illness and transiet stimulation of the parasympathetic nervous system was observed. The effect was not cumulative over the 3 week period. Recovery was prompt after cessation of test material. Larger doses given repetitively were lethal. No evidence of injury to the neuromuscular systems, or signs of peripheral neuritis, or loss of muscle control of demyelination in the peripheral nerves were observed. Trimethyl phosphate and triorthocresyl phosphate (positive controls) produced typical positive effects.

Potentiation Studies

Rats, female, weanlings 4/13/67, 6/12/67, 5/21/71
10 rats/group. 22 groups recievied the LD_{01} of each of 22 organophosphorous insecticides. 231 groups received binary mixtures containing the LD_{01} of each constituent insecticide. 571 groups received ternary mixtures containing the LD01 of each constituent insecticide. In 4/13/67 review, some potentiation was noted when Bidrin was used in combination with other O.P.s such as malathion, methyl parathion, Ronnel and Co-Ral. In other reviews, Bidrin potentiation was approximately 2-fold with Dimethoate and Guthion and essentially negative for the others.

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Mutagenic Study

Mice 1/14/76

A mutagenic study entitled "Toxicity studies with Bidrin: Chromosome studies on bone marrow cells of mice after a single oral dose of Bidrin" was submitted with this petition. See the section of this review entitled "New Studies Submitted in this Petition" for a review of this study.

Metabolism Studies

Rats, dogs, goats (oral) 5/21/71
mice, rabbits (i.p.)

The majority of administered radioactivity was excreted in the urine within 6 hours and about 63-71% within 48 hours. The major metabolite was N-methyl Bidrin (Azodrin). Milk from goats contained Azodrin in larger amounts than the parent compound Bidrin.

Rats 5/21/71

10 mg/kg of Bidrin - ^{32}P. After 2 hours, hydroxymethyl Bidrin and N-methyl Bidrin (Azodrin) represented a major fraction of the total compounds excreted. After 20 hours, 83% of recovered radioactivity was in the form of dimethylphosphate. After 6 hours, 65% of the total administered radioactivity was excreted.

Cows 5/21/71

0.25 mg/kg/day (about 6 ppm) in silage for 28 days. Urine was collected at 17-21 days and found to contain 0-0.2 ppm Bidrin and 0.13-0.27 ppm Azodrin. Milk and feces were negative for Bidrin and Azodrin.

Antidotal Studies	Review Date
Rats	5/21/71

Atropine methonitrate; and atropine sulfate; and P-2S; and atropine sulfate plus P-2S; and toxogonin; and atropine sulfate plus toxogonin each raised the acute subcutaneous LD_{50} of Bidrin. Atropine sulfate plus toxogonin was the most effective.

Guinea pigs, technical 5/21/71

Atropine; and atropine plus P-2S (2-hydroxyiminomethyl-N-methylpyridinium methane sulphonate) both decreased mortality in guinea pigs given 5 times the acute subcutaneous LD_{50} to the same degree (1/9 and 0/10 respectively). In animals given 10 times the LD_{50}, atropine plus P-2S was more effective (7/9 and 1/10 respectively).

Human Toxicity Studies	Review Date
Cholinesterase studies	5/21/71

Plasma and erythrocyte cholinesterase activities remained within the normal range in 29 persons applying Bidrin insecticide under practical field conditions.

Exposure studies 5/21/71

Based on residue exposure studies on 12 drivers and swampers operating applicators and on the pilot of one airplane swamper in the normal course of their duties, the total exposure to Bidrin for a 60 kg man was calculated to be 0.0016 mg/kg body weight.

New Studies Submitted in this Petition:

Two new studies were submitted in this petition - a teratology study and a mutagenic study.

Teratology Study

Identification of Study

"Toxicity studies with BIDRIN: Teratological studies in rabbits given BIDRIN orally," Expt. No. 291 and 374, Group Research Report TLGR.0020.73, August, 1973, Tunstall Laboratory and the Statistics Unit of Sittingbourne Laboratories.

Experimental Procedures

Technical Bidrin (Woodstock Agricultural Research Centre, ~~846.4%~~ 86.4% W/W of the cis isomer), as a 10% W/V solution in corn oil, was administered orally in gelatin capsules to mated female banded Dutch rabbits from day 6 to day 18 inclusive of gestation. Two separate experiments were run (Experiment A and Experiment B) but were reported together.

Experiment A

Test Material, Dose	Group	No. of does mated
vehicle only	Neg. Control	32
Bidrin, 1.3 mg/kg/day	Test	16
Bidrin, 4.0 mg/kg/day	Test	16
Thalidomide, 37.5 mg/kg/day	Pos. Control	16

Experiment B

Test Material, Dose	Group	No. of does mated
Vehicle only	Neg. Control	36
Bidrin, 1.3 mg/kg/day	Test	18
Bidrin, 4.0 mg/kg/day	Test	18
Bidrin, 8.0 mg/kg/day	Test	18
Thalidomide, 37.5 mg/kg/day	Pos. Control	18
Thalidomide, 75.0 mg/kg/day	Pos. Control	18

Note: Preliminary experiments had established the maximal tolerated dose of Bidrin to be about 4 mg/kg/day (by general health and body weights).

The general health and body weights of does were monitored. All does were killed at 28 days. Fetuses were removed by Caesarion section and externally examined. The number of live fetuses, late fetal deaths and resorption sites were determined. Live fetuses were placed in incubators for 24 hours and then were killed.

Fetal body weights and measurements were recorded and followed by examinations for skeletal, visceral and other abnormalities. In experiment A, about 2/3 of each litter was examined for skeletal abnormalities and about 1/3 for visceral abnormalities. In experiment B, all fetuses "were examined as completely as possible for skeletal and visceral defects". Statistical analyses were used where appropriate.

Results

Body Weights of Does

Expt. A - There was a tendency for all Bidrin - and especially the thalidomide - treated groups to gain less body weight than controls. Only one statistically significant ($p \leq 0.01$) difference occurred however (Bidrin, 1.3 mg/kg/day, at 15 days only). The effect did not appear to be dose-related.

Expt. B - Bidrin - treated animals (4 and 8 mg/kg/day) tended to gain less body weight than controls but no differences were statistically significant. Body weights for thalidomide-treated animals were significantly less than controls at $\geq$ 15 days.

General Health of Does

Expt. A - 1 female in each of the 4 groups tested aborted.

Expt. B - At 8 mg/kg/day (about double the maximal tolerated dose), severe effects due to the test material (including salivation and tremors) were observed and 4 animals died. One 37.5 mg/kg/day thalidomide animal also died. At $\leq$ 4 mg/kg/day, animals were unaffected. One animals aborted. (Bidrin, 1.3 mg/kg/day).

Effects on Pregnancy and the Fetus (non-teratogenic)

Expt. A and Expt. B - There were no statistically significant differences between control animals and Bidrin-or thalidomide-treated animals in the following parameters.

(1) number of abortions
(2) number of pregnancies surviving to term
(3) mean live litter sizes
(4) number of resorptions
(5) number of late fetal deaths
(6) size and weights of fetuses

24 hour survival of incubated live fetuses was significantly decreased in all thalidomide-treated groups. Survival in Bidrin-treated groups (with one unimportant exception) was comparable to controls.

Teratogenic Effects
Expt. A

Using litters as the experimental unit

Group	Skeletal Abnormalities pos./no. exam	%	Visceral Abnormalities pos./no. exam.	%
Neg. Control	4/24	16.7	0/24	0.0
Bidrin, 1.3 mg/kg/day	5/15	33.3	1/15	6.7
Bidrin, 4.0 mg/kg day	4/13	30.8	3/13	23.1*
Thalidomide, 37.5 mg/kg/day	7/12	58.3*	2/12	16.7

Using Fetuses as the experimental unit

Groups	Skeletal Abnormalities pos/no.exam.	%	Visceral Abnormalities pos./no.exam.	%
Neg. Control	4/54	7.4	0/54	0.0
Bidrin, 1.3 mg/kg/day	5/46	10.9	1/36	2.8
Bidrin, 4.0 mg/kg/day	8/34	23.5*	4/27	14.8*
Thalidomide, 37.5 mg/kg/day	12/30	40.0*	3/30	10.0*

*significantly higher than neg. controls ($p \leq 0.05$)

Expt. B

Using litters as the experimental unit

Group	Skeletal Abnormalities pos./no.exam.	%	Visceral Abnormalities pos./no.exam.	%
Neg. Control	6/21	28.6	2/21	9.5
Bidrin, 1.3 mg/kg/day	5/12	41.7	0/12	0.0
Bidrin, 4.0 mg/kg/day	1/13	7.7	0/13	0.0
Bidrin, 8.0 mg/kg/day	1/8	12.5	0/8	0.0
Thalidomide, 37.5 mg/kg/day	11/13	84.6*	6/13	46.2*
Thalidomide, 75.0 mg/kg/day	10/13	76.9*	6/13	46.2*

Using Fetuses as the experimental unit

Group	Skeletal Abnormalities pos./no.exam.	%	Visceral Abnormalities pos./no.exam.	%
Neg. Control	8/146	5.5	2/146	1.4
Bidrin, 1.3 mg/kg/day	5/80	6.3	0/80	0.0
Bidrin, 4.0 mg/kg/day	1/96	1.0	0/96	0.0
Bidrin, 8.0 mg/kg/day	2/53	3.8	0/53	0.0
Thalidomide, 37.5 mg/kg/day	26/88	29.5*	7/88	8.0*
Thalidomide, 75.0 mg/kg/day	28/76	36.8*	8/76	10.5*

*significantly higher than neg. controls ($p \leq 0.05$)

There were no statistically significant differences between neg. control animals and Bidrin - or thalidomide-treated animals in Expt. A or in Expt. B in the following parameters:

(1) delayed ossification
(2) extra pairs of ribs (bilateral)
(3) unilateral extra ribs.

Discussion

Skeletal Abnormalities

In Expt. A, a statistically significant ($p \leq 0.05$) increase in skeletal abnormalities was observed when fetuses were used as the experimental unit (at 4 mg/kg/day) but not when litters were used as the experimental unit (at 4 mg/kg/day). In Expt. B, statistically significant increases were not observed when either fetuses or litters were used as the experimental units (at doses up to and including 8 mg/kg/day). Statistically significant increases in thalidomide-treated animals (positive controls) were consistently observed in all thalidomide-treated groups in both Expt. A and Expt. B. The above data indicates that the single observed increase in skeletal abnormalities (using fetuses as the experimental unit, at 4 mg/kg/day in Expt. A) was probably a statistical artifact.

Visceral Abnormalities

In Expt. A, statistically significant ($p \leq 0.05$) increases in visceral abnormalities were observed when both fetuses and litters were used as the experimental unit (at 4 mg/kg/day). The incidence of visceral abnormalities in concurrent negative controls, however, was zero. In Expt. B, statistically significant increases were not observed when either fetuses or litters were used as the experimental units (at doses up to and including 8 mg/kg/day). In fact, no visceral abnormalities were observed in any of the Bidrin-treated animals whereas visceral abnormalities were observed in 2 negative control animals. Statistically significant increases in thalidomide-treated animals (positive controls) were consistently (with 1 exception-using litters as the experimental unit, at 37.5 mg/kg/day in Expt. A) observed in all thalidomide-treated groups in both Expt. A and Expt B.

The above data indicates that the observed increase in visceral abnormalities in Expt. A was probably a fortuitous artifact. The NEL for teratogenic effects, therefore, > 8 mg/kg/day (highest dosage level tested).

Mutagenic Study

Identification of Study

"Toxicity studies with Bidrin: Chromosome studies on bone marrow cells of mice after a single oral dose of Bidrin", Expt. No. 513, Group Research Report TLGR. 0040.73, December, 1973, Tunstall Laboratory.

Experimental procedure

Analytical grade Bidrin (≥ 99% active - Batch ACD/72/63) was administered orally to groups of 8 male and 8 female CF1 specific pathogen-free mice, aged 8-10 weeks, at 5 or at 10 mg/kg (about 1/4 and 1/2 the LD_{50}). Additional groups of control animals were dosed with water. One-half of the mice were sacrificed at 8 hours and one-half at 24 hours following administration of Bidrin or water. 0.01 ml/gm of 0.04% Colcemid solution was injected intraperitoneally at 90 min. before sacrifice (to arrest mitosis in vivo). Femurs were removed and chromosome preparations of bone marrow cells were prepared and examined for polyploidy, chromatid gaps, chromatid breaks and other chromosome aberrations (about 100 cells/animal).

Results

No significant differences in chromosomes abnormalities were demonstrated between treated and control animals.

Summary of Background Toxicity Data and of New Studies Submitted in this Petition:

Acute Oral Toxicity

Rats, techncial	LD_{50}=22(19-25) mg/kg
Rats, technical	LD_{50}=12.8(9.1-15.7)mg/kg
Rats, in isopropyl alcohol	LD_{50}-28.7 mg/kg
Rats, in dimethyl sulphoxide	LD_{50}=30.2 mg/kg
Mice, male	LD_{50}=15 mg/kg
Mice, in isopropyl alcohol	LD_{50}=20 mg/kg
Mice, in dimethyl sulphoxide	LD_{50}=40-50 mg/kg
Ducks	LD_{50}=2.5-5.0 mg/kg
Fowls	LD_{50}=10-12.5 mg/kg
Hens	LD_{50}=7.4 mg/kg

Acute Dermal Toxicity

Rats, male, technical	LD_{50}=136 mg/kg
Rats, female, technical	LD_{50}=111 mg/kg
Rabbits	LD_{50}=224 (102-505) mg/kg (6 hour exposure)

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Cattle
60 mg/kg (0.5% spray) to head-borderline systemic toxicity
60 mg/kg (in water or glycerin) to back - no symptoms
12.5 mg/kg (in olive oil) to back-definite systemic toxicity
0.25% spray (full coverage) - no symptoms

Acute Inhalation Toxicity	
Rats, male, technical, undiluted	LC_{50}=0.65 mg/l (1 hour exposure)
Rats, male, 38.2% Bidrin, aqueous	LC_{20} = 0.86 mg/l (not lethal at 2 mg/soln/l)
Acute Subcutaneous Toxicity	
Guinea pigs, technical	LD_{50}=45 mg/kg
Primary Eye Irritation	
Rabbits	conjunctival erythema (grades 2-3), opacity (grade 2), discharge (grade 1). Normal within 24 hours.
Primary Skin Irritation	
None	
Subacute Dermal Toxicity	
Rabbits	0, 20 and 40 mg/kg/day for 3 weeks. 2 mortalities at 40 mg/kg/day. Very mild skin irritation in a few animals, majority showed no dermal irritation.
Subacute Inhalation Toxicity	
Rats	NEL=0.81 mg/l.
Subacute Feeding Toxicity	
Rats, 2 weeks	Subacute LD_{50} = 440 ppm
Rats, 12 weeks	NEL=45 ppm (systemic effects only)
Rats, 12 weeks	NEL=0.5 ppm (cholinesterase effects only)
Dogs, 3 1/2 weeks	NEL=1.5 ppm (cholinesterase effects only)
Dogs, 13 weeks	NEL < 1 ppm (cholinesterase effects) NEL = 50 ppm (systemic effects)

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Chronic Feeding Toxicity
Rats, 2 years — NEL=1 ppm (cholinesterase effects); NEL=10 ppm (systemic effects)
Dogs, 2 years — NEL=1.6 ppm (cholinesterase effects); NEL=16 ppm (systemic effects)

Oncogenic Study
Rats, 2 years

The 2 year chronic feeding study on rats described above includes a valid oncogenic evaluation. Results for oncogenicity were negative.

Reproduction Studies
Rats, one generation — NEL=3.0 ppm (systemic effects)
Rats, three generations — NEL=2.0 ppm (systemic effects)

Teratology Studies
Rats — Up to 100 ppm in diet. No terata. "It is suggested that Bidrin may interfere with implantation and placentation."
Rabbits — Up to 8.0 mg/kg/day (orally) from day 6 to day 18 of gestation. Significant differences in number of terata were observed between test and control animals in first part of study (Expt. A) but were satisfactorily accounted for as statistical artifacts in second part of study (Expt.B). See review for details. NEL > 8 mg/kg/day (highest dosage level tested).

Neurotoxicity Studies

Rats, 6 week feeding study NEL < 5 ppm (cholinesterase effects)
Dogs, 12 week feeding study NEL = 1.5 ppm (cholinesterase effects)
Hens, demyelination study, single oral doses

No neurotoxicity at 8-9 ~~m/kgf~~. mg/kg.

Hens, demyelination study, oral administration of 1/10 LD_{50} "on each of several days" for 3 weeks. Negative for neurotoxicity.

Potentiation Studies
Rats, female, weanlings
Some potentiation of Bidrin when administered in combination with malathion, methyl parathion, Ronnel, Co-Ral, dimethoate and Guthion.

Mutagenic Study

Mice — Chromosome preparations of bone marrow cells from mice given single oral administrations of Bidrin (5 and 10 mg/kg) were examined. No significant differences in chromosome abnormalities were demonstrated between treated and control animals.

Metabolism Studies

Rats, dogs, goats (oral)
mice, rabbits (i.p.)

Most administered radioactivity (63-71%) excreted in urine within 48 hours. Major metabolite was N-methyl Bidrin.

Rats

2 hours after administration of Bidrin-^{32}P, hydroxymethyl Bidrin and N-methyl Bidrin were major metabolites. After 20 hours, 83% of recovered radioactivity was in the form of dimethylphosphate.

Cows

About 6 ppm in silage for 28 days. Urine contained 0-0.2 ppm Bidrin and 0.13-0.27 ppm N-methyl Bidrin. Milk and feces were negative.

Antidotal studies

Rats

Atropine, P-2S and toxogonin alone and in various combinations raised the subcutaneous LD_{50} of Bidrin.

Guinea pigs

Atropine and P-2S both decreased the mortality in guinea pigs given 5 times and 10 times the acute subcutaneous LD_{50}.

Human Toxicity Studies

Plasma and erythrocyte cholinesterase activities remained within the normal range in 29 persons applying Bidrin under practical field conditions.

Based on residue exposure studies on 12 drivers and swampers and on the pilot of an airplane swamper in the normal course of their duties, the total exposure to Bidrin for a 60 kg man was calculated to be 0.0016 mg/kg body weight.

Present Action:

In this petition the petitioner has requested the establishment of a tolerance for residues of the insecticide dimethyl phosphate of 3-hydroxy-N,N-dimethyl-cis-crotonamide in or on wheat and sorghum grain of 0.05 ppm.

A careful review of all data submitted and referenced by the petitioner has disclosed the following deficiency in basic toxicological data requirements for tolerances as described in exhibit 1 of the memo from the Pesticide Science Officer to the Branch Chief of Toxicology (dated June 25, 1976):

(1) oncogenic potential evaluation in two mammalion species. The 2 year chronic feeding study on rats is acceptable as a valid oncogenic evaluation. An oncogenic evaluation in a second mammalian species, however, has not been submitted or referenced.

Due to the deficiency listed above, Toxicology Branch must recommend against establishment of the requested tolerance at this time.

Edwin R. Budd 2/4/77

Edwin R. Budd, Toxicologist
Toxicology Branch
Registration Division (WH-567)

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Chemical:	**Dimethyl phosphate ester with 3-hydroxy-**
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HED File Code	**13000 Tox Reviews**
Memo Date:	**02/04/77**
File ID:	**TX000094**
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